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EXAMINER
SLOBODYANSKY, ELIZABETH

ART UNIT	PAPER NUMBER
1652	14

DATE MAILED: 07/09/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/852,000	OSUMI ET AL.	
	Examiner	Art Unit	
	Elizabeth Slobodyansky	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 April 2003* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *8,9,11-14 and 16-20* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *8,9,11-14 and 16-20* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *21 April 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

The amendment filed April 21, 2003 amending the specification to insert reference to the sequence identifiers, canceling claims 10 and 15 and amending claims 9, 12, 14, 17 and 19 has been entered.

Claims 8, 9, 11-14 and 16-20 are pending.

Drawings

The new drawings filed April 21, 2003 have been approved by Draftsman.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 8, 11, 13, 16, with dependent claims 17-20, are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 8, 11, 13 and 16 are directed to a DNA encoding a fluorescent protein comprising the amino acid sequence set forth in SEQ ID NO:1 with at least mutations of

Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; and Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly, respectively. Claims 17-20 are dependent claims and are drawn to methods of use thereof.

The specific recited mutations constitute no more than 2.9% of the entire SEQ ID NO:1 that is 238 amino acids long. The use of "at least mutations" renders the claims to encompass DNAs encoding a fluorescent protein having any structure and any fluorescent characteristics as long as its structure comprises the above mutations. Therefore, the claims are drawn to a genus of DNAs encoding fluorescent proteins described by insufficient limitations on either structure or function. The specification discloses no identifying characteristics which would allow to recognize a structure as exhibiting any fluorescence.

The specification teaches the representative species of such DNAs encoding the amino acid sequences that differ from SEQ ID NO:1 by only the recited mutations with the rest of SEQ ID NO:1 intact. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of encoding a fluorescent protein.

Therefore, based on the instant disclosure, it is unpredictable either a protein is a fluorescent protein. Thus, a DNA encoding a fluorescent protein comprising the amino acid sequence set forth in SEQ ID NO:1 with at least mutations of Phe64Leu,

Val163Ala and Ser175Gly; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; or Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly, lacks sufficient written description needed to practice the invention of claims 8, 11, 13 and 16-20.

Claims 8, 11, 13 and 16-20 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a DNA encoding a fluorescent protein comprising the amino acid sequence that differs from SEQ ID NO:1 by only mutations Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; or Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly, does not reasonably provide enablement for a DNA encoding a fluorescent protein having the amino acid sequence of SEQ ID NO:1 with at least mutations Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; or Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir.

1988). They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7)considered in determining whether undue experimentation is required, are summarized the predictability or unpredictability of the art, and (8) the breadth of the claims.

Claims 8, 11, 13 and 16-20 are directed to or depend from a DNA encoding a fluorescent protein comprising the amino acid sequence set forth in SEQ ID NO:1 with at least mutations Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; or Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly. This amounts to a DNA encoding any fluorescent protein having any structure and any fluorescent characteristics as long as its structure comprises the above mutations.

The specification does not support the broad scope of the claims which encompass all modifications and fragments of any sequence that comprises mutations Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; or Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly because the specification does **not** establish: (A) regions of the protein structure which may be modified without effecting the specific requisite activity of the polypeptide of the instant invention; (B) the general

tolerance of said polypeptide to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residues with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Despite knowledge in the art to produce mutations in proteins, the specification fails to provide guidance as to where, and what type of (i.e., what amino acid to substitute into, add to or delete from the known sequence), changes in amino acid residues will result in a desired biological activity. The amino acid sequence of a protein determines its structural and functional properties, and predictability of what mutations can be tolerated in a protein's sequence and result in a certain activity is extremely complex, and well outside the realm of routine experimentation, because accurate predictions of a protein's function from mere sequence data are limited.

Furthermore, while recombinant and mutagenesis techniques are known, it is not routine in the art to screen large numbers of mutated proteins where the expectation of obtaining similar activity is unpredictable based on the instant disclosure.

Therefore, one of ordinary skill in the art would require guidance, in order to make a DNA encoding a fluorescent protein having any amino acid sequence of unknown homology to SEQ ID NO:1 with at least mutations Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His,

Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; or Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly in a manner reasonably correlated with the scope of the claims. Without such guidance, the experimentation left to those skilled in the art is undue.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 9, 12 and 14, with dependent claims 17-20, are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Amended claims 9, 12 and 14 recite "SEQ ID No.1, said sequence consisting of the [ ] mutations". The claims are confusing because the claimed sequences do not anymore have the sequence of SEQ ID NO:1 and SEQ ID NO:1 does not consists of the recited mutations. Amending the claims to recite "DNA encoding a fluorescent protein comprising the amino acid sequence that differs from SEQ ID NO:1 by only mutations ...", for example, would obviate this rejection.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be

> patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 8 and 9, with dependent claims 17 and 18, are rejected under 35 U.S.C. 103(a) as being unpatentable over Siemering et al. in view of Thastrup et al.

Siemering et al. (form PTO-1449 filed May 10, 2001, reference 12) discloses a DNA encoding a GFP mutant (GFPA) comprising double mutation Val163Ala/Ser175Gly (page 1654, 2nd column). They teach that said mutant has about 4 fold higher fluorescence at 37°C than at 30°C (page 1655, figure 2).

Thastrup et al. (WO 97/11094, form PTO-1449 filed May 10, 2001, reference 6) teach mutant GFP proteins comprising mutation F64L such as F64L-GFP, F64L-Y66H-GFP and F64L-S65T-GFP that have a cellular fluorescence far exceeding the cellular fluorescence of the parent proteins (page 3, lines 12-15). They teach that said proteins have a higher fluorescence at 37°C than at 22°C (Figures).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine mutations Val163Ala/Ser175Gly taught by Siemering et al. with F64L mutation taught by Thastrup et al. One skilled in the art would have been motivated to combine these mutations on order to make a mutant having an improved fluorescence at higher temperatures. One could have a reasonable expectations of success of at least accumulative effect because both mutants retain their respective properties when combined with other mutations.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a cell transfected with a DNA encoding the fluorescent mutant for visually analyzing gene expression or protein localization according to the intended use of such mutants as taught by Siemering et al. and Thastrup et al.

Because the V163A/S175G GFP mutant alone has about 4 fold higher fluorescence at 37°C than at 30°C (Siemering et al., *supra*), the fluorescence exhibited by the 105 mutant (Phe64Leu/Val163Ala/Ser175Gly) that is about 3.5 times higher fluorescence at 37°C than at 30°C is not deemed as an unexpected result (page 31, Table 5).

Claim 16, with dependent claims 19 and 20, is rejected under 35 U.S.C. 103(a) as being unpatentable over Tsien et al. in view of Siemering et al.

Tsien et al. (US Patent 6,197,928, form PTO-1449 filed July 10, 2002) teach a DNA encoding the "blue" fluorescent GFP mutant P4-3 comprising mutations Y66H/Y145F (column 10, Table 1). They teach that it has dim fluorescence at 37°C (column 23, lines 15-21).

The teachings of Siemering et al. are outlined above.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine P4-3 mutations taught by Tsien et al. with Val163Ala/Ser175Gly taught by Siemering et al. which are responsible for a higher

fluorescence at 37°C than at 30°C. One skilled in the art would be motivated to combine these mutations on order to make a BFP with improved fluorescence at higher temperatures. One could have a reasonable expectations of success of an accumulative effect because both mutants, Y66H/Y145F taught by Tsien et al. and Val163Ala/Ser175Gly taught by Siemering et al., retain their respective properties when combined with other mutations.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a cell transfected with a DNA encoding the fluorescent mutant for visually analyzing gene expression or protein localization according to the intended use of such mutants as taught by Tsien et al. and Siemering et al.

Allowable Subject Matter

Claims 12 and 14 would be allowable if rewritten to overcome the rejection(s) under 35 U.S.C. 112, second paragraph, set forth in this Office action and to include all of the limitations of the base claim and any intervening claims.

Response to Arguments

Applicant's arguments filed April 21, 2003 have been fully considered but they are not persuasive.

With regards to the written description rejection Applicants argue that "this [residues 65-66-67 of SEQ ID NO:1] grouping of amino acids forms identifiable structure disclosed in the application as contributing to fluorescence (Remarks, page 6, penultimate paragraph). This is not persuasive because while the discussed triad is contributing to fluorescence, it is not fluorescent itself. Therefore, the correlation between the structure and fluorescence common to all members of the genus is lacking from the description. Furthermore, the triad essential to fluorescence is known in the art and by itself does not distinguish the claimed genus from the prior art.

Applicants further argue that they "disclose actual reduction to practice of at least two mutants - BFP(202) and BFP(205) - having the recited mutations [Phe64Leu/ Tyr66His/Tyr145Phe]" (paragraph bridging page 7 and 8). The disclosed mutants (either BFP(202) or BFP(205)) do not support sufficient written description of the genus because the mutations represent a minuscule percent of the structure that by itself is insufficient to impart fluorescence. There is no description of the rest of the protein structure because allowed mutations in SEQ ID NO:1 are not limited to the mutations present in the disclosed mutants. Applicants recite the Guidelines as follows "what constitutes a 'representative number' [of species] is an inverse function of the skill in the art [and] depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus". Applicants continue with the

reference to the Guidelines that "there is no basis for a *per se* rule requiring disclosure of complete DNA sequences or limiting DNA claims to only the sequence[s] disclosed" (page 9, 1st paragraph). Applicants argue that "in view of the actual reduction to practice of several species within the claimed genus, the functional correlation between the structure of these mutants and a higher fluorescence, and the fact that the skill in the art of GFP mutations is well developed such that the skilled artisan would immediately envision a genus of mutants having the recited mutations, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph, for alleged lack of written description" (page 10). This is not persuasive because the examiner did not state that claims should be limited to only the disclosed sequences. However, the examiner is not persuaded that one of skill in the art "would immediately envision a genus of mutants having the recited mutations" other than a genus of mutants having the recited mutations when said genus comprises sequences that are highly homologous to SEQ ID NO:1. Mutants BFP(202) or BFP(205) are examples of such sequences. However, the rejected claims are not limited to a highly homologous genus. The claimed sequences may differ in more than 95% of their structures. Neither art nor the teachings of the specification allows to envision sequences with unknown, possibly low, degree of homology to SEQ ID NO:1 comprising the recited mutations and exhibiting the fluorescence.

With regard to the enablement rejection, Applicants argue that "the specification discloses more than one mutants falling within the scope of each claim" (page 11, 1st paragraph). Applicants assert that "the isolation of more than one protein within the claimed genus provides an expectation that one of skill in the art may successfully identify further mutant proteins within the genus using the methods disclosed in the specification. Indeed, the Application discloses at pages 18-20 a method for randomly introducing mutations into a GFP sequence using Mutagenic PCR, wherein random mutants are screened for increased fluorescence in *E. coli* following UV irradiation" (page 11, 2nd paragraph). This is not persuasive because while methods to produce variants of a known sequence such as Mutagenic PCR are well known to the skilled artisan, producing variants as claimed by applicants (i.e., DNA encoding a fluorescent protein of an unknown homology to SEQ ID NO:1 comprising the specific mutations) requires that one of ordinary skill in the art know or be provided with guidance for the selection of which of the infinite number of variants have the claimed property. Without such guidance one of ordinary skill would be reduced to the necessity of producing and testing all of the virtually infinite possibilities. This would clearly constitute **undue** experimentation. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has **not** been provided in the instant

specification. As previously stated the specification does not establish: (A) regions of the protein structure (SEQ ID NO:1) which may be modified without effecting the fluorescence; (B) the general tolerance of the protein stricture (SEQ ID NO:1) to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any residues with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

With regard to Applicants' references to U.S. Patent 6,194,548 (pages 10, 13), the examiner notes that the instant application not the issued patent is currently under consideration.

With regard to the 103(a) rejection of claims 8, 9, 17 and 18, Applicants argue that "were the teachings of two or more prior art references conflict, the Examiner must weigh the power of each reference to suggest solutions to one of ordinary skill in the art, considering the degree to which one reference might accurately discredit another" (page 15, 1st full paragraph, emphasis added). They refer to EBFP mutant as discussed in Angres et al. (1999) and Ellenberg et al. (November 1998), both cited in IDS filed July 10, 2002. This is not persuasive because the cited references are not prior art. Furthermore, while EBFP produced by Clontech may have some drawbacks, it has commercial success lasting up to date. With regard to the 103(a) rejection of claims 16, 19 and 20, Applicants similarly argue that if EBFP has problems, so should

other mutants comprising mutations Tyr66His and Tyr145Phe (page 17). This is not persuasive in view of the above discussion of the art. Furthermore, EBFP comprises mutations other than recited in claim 16 and each mutant is a distinct compound with its own line of consideration.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Elizabeth Slobodyansky whose telephone number is (703) 306-3222. The examiner can normally be reached Monday through Friday from 9:30 AM to 6:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX phone number for Technology Center 1600 is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Center receptionist whose telephone number is (703) 308-0196.

E. Slobodyansky

Elizabeth Slobodyansky, PhD
Primary Examiner

July 7, 2003